
PHYLOGENETIC RELATIONSHIPS *Gretchen M. Ionta² and Walter S. Judd²* IN PERIPLOCOIDEAE (APOCYNACEAE S.L.) AND INSIGHTS INTO THE ORIGIN OF POLLINIA¹

ABSTRACT

Phylogenetic relationships within Periplocoideae (Apocynaceae s.l.) were investigated using sequence data from nuclear ribosomal DNA and two plastid regions: *trnD-trnT* and *trnT-trnF*. We included 43 ingroup taxa, representing all but three of 32 currently recognized genera. In the ITS and total evidence analyses, *Phyllanthera grayi* (P. I. Forst.) Venter is supported as sister to the rest of the subfamily. We identified several well-supported clades, and three of six genera represented here by more than one species (*Cryptolepis* R. Br., *Pentopetia* Decne., and *Periploca* L.) are supported as monophyletic. Pollinial species appear in four distinct, well-defined clades, suggesting either three or four independent origins of pollinia within the subfamily. It is most parsimonious, therefore, to view pollinia in Periplocoideae as non-homologous with those of Secamonoideae and Asclepiadoideae, a hypothesis that is supported by their structural differences.

Key words: Apocynaceae, character evolution, molecular phylogeny, Periplocoideae, pollinium, tetrad, translator.

Periplocoideae comprise ca. 190 species in 32 genera found throughout the Old World tropics and subtropics and centered in Africa, Asia, and Madagascar; some species occur in temperate regions and a few are found in Australia and Europe. The majority are woody climbers occupying tropical and subtropical forests; other forms include erect or straggling shrubs from semi-arid and desert regions, suffrutescent forest climbers, herbaceous geophytes from open habitats, and epiphytes in tropical forests (Venter, 1997; Venter & Verhoeven, 1997, 2001). The generally small (most under 10 mm long) yet extremely complex flowers of Periplocoideae exhibit the high degree of synorganization between androecium and gynoecium typical for advanced members of Apocynaceae s.l. Floral form varies among as well as within genera. This is evidenced by a wide range of corolla shapes, an elaborate diversification of corolline coronas, varying degrees of fusion between coronas and stamens, and differences in the position of floral organs on the corolla tube. Venter and Verhoeven (1997, 2001) provide an excellent review of the range of vegetative and floral diversity present in Periplocoideae.

Over the years Periplocoideae have been included in Asclepiadaceae (Brown, 1810; Bentham, 1876; Schumann, 1895; Liede & Albers, 1994), considered a separate family (Schlechter, 1905; Bullock, 1956; Kunze, 1993; Omlor, 1996; Swarupananadan et al., 1996), or recognized as part of a more inclusive Apocynaceae along with other Asclepiadaceae (Safwat, 1962; Judd et al., 1994; Sennblad & Bremer, 1996; Venter & Verhoeven, 1997; Endress & Bruyns, 2000; Venter & Verhoeven, 2001). Brown (1810) included Periplocoideae, Asclepiadoideae, and Secamonoideae in Asclepiadaceae on the basis of morphological similarities, primarily the presence of translators (complex pollen carriers formed from hardened secretions of the styler head) in these taxa. The relatively soft translators of Periplocoideae consist of a spoon-shaped receptacle onto which pollen is shed at anthesis, an adhesive disc that attaches to the pollinator, and a stipe or stalk connecting the two (Safwat, 1962; Schick, 1982; Verhoeven & Venter, 1997). In most taxa, pollen is shed as separate tetrads; seven genera have been documented as having tetrads aggregated into pollinia (Verhoeven & Venter, 1998a, b, 2001, 2002). In

¹ This research would not have been possible without the generosity of many people and institutions. We thank Lucille Allorge, Peter Bruyns, Laure Civeyrel, Mary Endress, Paul Forster, Sigrid Liede, Tatyana Livshultz, Ulrich Meve, David Middleton, Clint Morse, Kent Perkins, and Johan Venter for providing plant material, and Norris Williams and Mark Whitten for assistance in the molecular lab and with phylogenetic analyses. A Deep Time grant funded herbarium research in Europe. We are grateful to Victoria Hollowell, David Middleton, and Mark Fishbein, whose comments greatly improved the manuscript. Gretchen Ionta personally thanks the staff of K, L, P, and Z for facilitating her research while in Europe; Lucille Allorge, Peter and Mary Endress, Sigrid Liede, Ulrich Meve, and Laure Civeyrel for their generous hospitality during her visit to Europe; and Barbara Carlsward for technical assistance with computers and microscopy. Last but certainly not least, a special thank you to Mary Endress for her advice and support during all stages of this study.

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Asclepiadoideae and Secamonoideae, single pollen grains or tetrads are aggregated into pollinia that are firmly attached to hardened, clip-like translators before anthesis. These differences in pollen and translator morphology led Schlechter (1905) to recognize Periplocaceae as a separate family. Today, most researchers agree that Periplocoideae, Asclepiadoideae, and Secamonoideae belong in an expanded Apocynaceae s.l. A number of phylogenetic analyses support this grouping, as well as the monophyly of Periplocoideae (Sennblad & Bremer, 1996; Civeyrel et al., 1998; Potgieter & Albert, 2001; Lahaye et al., 2005), and recent studies show that Periplocoideae are likely nested within the paraphyletic grade Apocynoideae (Sennblad & Bremer, 1996; Potgieter & Albert, 2001).

The number of accepted genera in Periplocoideae has changed considerably through the years and is still in flux. Given the small size of the subfamily, a disproportionately high number of monotypic and ditypic genera have been named. This may be due to a variety of factors: the group is extremely diverse, closely related species may differ greatly in morphology, many taxa (particularly in Asia) have not been extensively collected and are poorly known (Venter & Verhoeven, 1997; Meve & Liede, 2004), and the small yet highly complex flowers are difficult to interpret from herbarium material. A total of 86 genera have been named in Periplocoideae (Venter & Verhoeven, 1997). Venter and Verhoeven proposed a tribal classification of the subfamily in 1997, which included 44 genera in three tribes. Since then, a number of smaller genera were placed in synonymy by Klackenberg (1997, 1998, 1999) and Venter and Verhoeven (2001), reducing the number of genera to 31. Today approximately half of the species belong to one of three large genera: *Raphionacme* Harv. (37 species), *Cryptolepis* R. Br. (27), and *Pentopetia* Decne. (21). The remaining genera contain from one to 14 species; nine are monotypic and eight ditypic. Here we follow the generic circumscription of Venter and Verhoeven (2001) with one exception: in accordance with Meve and Liede (2004), we recognize the African *Petopentia natalensis* (Schltr.) Bullock as separate from the Malagasy endemic genus *Ischnolepis* Jum. & H. Perrier.

In his publications, N. E. Brown (1902, 1904) used variations in the length of the upper corolla tube to delimit genera within the subfamily. Venter and Verhoeven (1997) also emphasized floral features in a tribal classification of Periplocoideae based on overall corolla form, exposure of the gynostegium from the corolla, and the position of coronas and stamens in the corolla tube. A subsequent phylogenetic analysis of the subfamily by Venter and Verhoeven (2001) based on morphological data did not support the tribes proposed

in their earlier classification, and they noted that, within Periplocoideae, floral features such as the length of the corolla tube were more variable than previously thought.

Of the five subfamilies that form Apocynaceae s.l., Periplocoideae are unique in having taxa both with and without pollinia. Pollinia have been documented in seven genera: five Asian (*Decalepis* Wight & Arn., *Finlaysonia* Wall., *Gymnanthera* R. Br., *Hemidesmus* R. Br., and *Streptocaulon* Wight & Arn.) and two African (*Epistemma* D. V. Field & J. B. Hall and *Schlechterella* K. Schum.). Pollinia in Periplocoideae differ markedly from those found in other Apocynaceae: they are shed onto the translator at anthesis (in Secamonoideae and Asclepiadoideae the pollinia are firmly attached to hardened corpuscles before being removed from the anther sacs by pollinators), they lack the pollinium walls (ectexine) of Asclepiadoideae, and the tetrads that form a pollinium are only loosely agglutinated. Venter and Verhoeven (1997) hypothesized that pollinia in Periplocoideae evolved polyphyletically. Later (Venter & Verhoeven, 2001), they predicted an independent origin of pollinia in Asian and African Periplocoideae due to differences in the pollinium structure of *Schlechterella* as compared to that of other pollinial taxa that had been documented by them (Verhoeven & Venter, 1998b). Fishbein (2001: 609) explored the origins and diversification of pollinia in the former Asclepiadaceae, concluding that “pollinia of Periplocoideae are not reconstructed as homologous with those of other asclepiads. . . it appears that pollinia may have evolved independently on several occasions in Periplocoideae, based on the distribution of pollinia among putatively isolated genera in each of the three recognized tribes.”

Our primary goal in undertaking this study was to elucidate phylogenetic relationships within Periplocoideae. To that end, we utilized the ITS regions of nuclear ribosomal DNA and two plastid regions: *trnT-trnF* (the *trnT-trnL* intergenic spacer, the *trnL-trnF* spacer, and the *trnL* intron) and the *trnD-trnT* spacer. The *trnT-trnF* region (in part or in whole) has been used successfully to assess relationships among genera throughout Apocynaceae s. str. (Liede, 2001; Meve & Liede, 2001, 2002, 2004; Lahaye et al., 2005). The resulting hypotheses of phylogenetic relationships are compared with the tribes of Venter and Verhoeven (1997) and the results of former phylogenetic analyses.

MATERIAL AND METHODS

TAXON SAMPLING

Forty-five taxa representing 29 of 32 currently recognized Periplocoideae genera (sensu Venter &

Verhoeven, 2001, plus *Petopentia* Bullock) were included in this study, along with two outgroup taxa (Table 1). Outgroup choice of *Forsteronia leptocarpa* A. DC. and *Rhabdadenia biflora* Müll. Arg. was based on recent molecular studies nesting Periplocoideae within Apocynoideae (Sennblad & Bremer, 1996; Potgieter & Albert, 2001). We were unable to obtain sufficient material for DNA extraction of three genera: *Sarcorrhiza* Bullock, *Telectadium* Baill., and *Maclaudia* Venter & R. L. Verh.

Leaf tissue was obtained from live plants grown in the University of Florida Department of Botany greenhouse, from field-collected specimens dried in silica (generously donated by a number of researchers, see acknowledgments) and from herbarium material. Genomic DNA was isolated using a modification of the CTAB extraction protocol outlined by Doyle and Doyle (1987). Extracted DNA was purified using Qiagen QIAQuick columns (Qiagen, Santa Clarita, California, U.S.A.) using the manufacturer's protocol.

PCR AMPLIFICATION AND DNA SEQUENCING

Ribosomal DNA was amplified in 50- μ l reactions with primers 17SE and 26SE of Sun et al. (1994), using a touchdown thermal cycling program with an initial annealing temperature of 76°C, decreasing 1°C per cycle for 15 cycles, followed by 15 cycles at 61°C. With difficult samples (e.g., herbarium specimens), ITS-1 and ITS-2 were amplified separately using the primers and protocol of Blattner (1999).

The *trnD-trnT* intergenic spacer region was amplified and sequenced using the primers and protocol of Demesure et al. (1995). The *trnT-trnF* chloroplast region spans *trnT-trnL*, *trnL-trnF*, and the *trnL* intron. We amplified and sequenced this region using the primers and protocol of Taberlet et al. (1991), with the exception of their trnTR primer, which we substituted with trnT2F of Shaw et al. (2005). Attempts to amplify chloroplast regions from genomic DNA extracted from herbarium specimens often proved unsuccessful, resulting in fewer exemplars in the chloroplast region matrices.

PCR products were purified using Qiagen QIAQuick columns (Qiagen) using the manufacturer's protocol. Sequencing reactions were performed using Big Dye V3.1 chemistry. Primers used for cycle sequencing were the same as those used in PCR amplification. All sequencing was done on Applied Biosystems ABI 377 Sequencers (Applied Biosystems, Foster City, California, U.S.A.) at the Interdisciplinary Center for Biotechnology Research, University of Florida. In addition to the sequences generated for this study, we included sequences previously published by Meve and Liede (2004).

PHYLOGENETIC ANALYSES

Sequences were manually edited and assembled using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.), and consensus sequences were visually aligned using SeAl 2.0 (Rambaut, 1996). Regions of ambiguous alignment were identified and excluded from analyses. Parsimony analyses were conducted using PAUP* 4.0b10 (Swofford, 1999). For each heuristic search all characters were equally weighted, character state transitions were treated as unordered, and gaps were treated as missing data. Each matrix underwent 1000 random addition replicates, saving 10 trees per replicate, with tree bisection-reconnection (TBR) branch swapping, and with the MULTREES option in effect. The shortest trees retained in memory were then swapped to completion. Relative support for clades was evaluated using 1000 bootstrap (BS) replicates (Felsenstein, 1985), with 10 random addition replicates and subtree pruning and re-grafting (SPR) branch swapping.

Congruence of separate data sets was assessed using a conditional combination approach (Huelsenbeck et al., 1996), as described by Soltis et al. (2001). First, each data set was analyzed separately. We then compared the resultant topologies and searched for instances of strongly supported contradictory clades. Following the approach of Mason-Gamer and Kellogg (1996), we established a bootstrap value cutoff of 70% for assessing strong conflict between data sets. The chloroplast is inherited as a single unit and is not subject to recombination; therefore, we treated the plastid data sets as a single linkage partition. Congruence of nuclear and plastid data was further assessed using a partition homogeneity test, as implemented in PAUP* 4.0b10.

Analyses of the following data sets were performed: (1) ITS alone, comprising 43 ingroup taxa in 29 genera and two outgroup taxa; (2) *trnT-trnF*, with 27 ingroup taxa in 18 genera and two outgroup taxa; (3) the *trnD-trnT* region, with 21 ingroup taxa in 17 genera and one outgroup taxon; (4) a combined chloroplast data set containing 28 ingroup taxa in 19 genera and two outgroup taxa; and (5) a combined total evidence data set of 45 taxa (43 ingroup taxa in 29 genera and two outgroup taxa). Chloroplast sequences are missing for several taxa, and as a result the data sets for analyses (4) and (5) are incomplete; missing sequences are indicated by dashes in Table 1.

RESULTS

ITS

The aligned ITS matrix was 774 characters long; 265 of 399 variable characters were parsimony

informative. Parsimony analysis resulted in 93 most parsimonious trees (MPT) of 1223 steps (consistency index [CI] = 0.474; retention index [RI] = 0.609; rescaled consistency index [RC] = 0.289); the strict consensus of these trees is shown in Figure 1. *Phyllanthera grayi* (P. I. Forst.) Venter, an Asian taxon, is supported as sister to the rest of the subfamily (BS 99). A large, well-supported clade (BS 90; herein referred to as the grooved translator clade) contains 23 Asian and African taxa, including the eight pollinial taxa included in this analysis. Within this clade, there is moderate support (BS 72) for an Asian clade of seven taxa (the Asian pollinial clade), the majority of which (five of seven) bear pollinia. Within this Asian pollinial clade, the monophyly of *Finlaysonia*, represented in this study by *F. insularum* (King & Gamble) Venter and *F. lanuginosa* (Ridl.) Venter, is not supported. *Finlaysonia lanuginosa* is sister to *Streptocaulon juvenas* (Lour.) Merr. (BS 71), while *F. insularum* forms a clade with *Atherandra acuminata* Decne.–*Gymnanthera oblonga* (Burm. f.) P. S. Green–*Zygotelma benthamii* Baill. (BS 89). Sister to this Asian pollinial clade (BS 95) is a clade containing two African taxa: *Buckollia volubilis* (Schltr.) Venter & R. L. Verh. (tetrads) and *Epistemma rupestre* H. Huber (pollinia-bearing). *Raphionacme* is paraphyletic, with all but one species forming a clade (BS 100); the exception, *Raphionacme lobulata* Venter & R. L. Verh., is sister to the genus *Stomatostemma* N. E. Br. (BS 59). Four of five *Periploca* L. species included in this analysis form a clade (BS 100); the exception, *P. nigrescens* Afzel., is sister to *Omphalogonus calophyllus* Baill. (BS 100). *Cryptolepis* and *Pentopetia*, relatively large genera represented here by five of 27 species and two of 21 species, respectively, are both supported as monophyletic (BS 99, BS 97).

PLASTID REGIONS

The aligned *trnT-trnF* matrix was 1757 characters long after removal of sites where alignment was ambiguous; 57 of 231 variable characters were parsimony informative. Parsimony analysis yielded 9890 shortest trees of 262 steps (CI = 0.931; RI = 0.841; RC = 0.783). The aligned *trnD-trnT* matrix was 1292 characters long after removal of sites where alignment was ambiguous; 20 of 194 variable characters were parsimony informative. Parsimony analysis yielded 9010 shortest trees of 212 steps (CI = 0.948; RI = 0.667; RC = 0.632).

Visual inspection of the strict consensus trees from the separate *trnT-trnF* and *trnD-trnT* analyses revealed no strongly supported contradictory clades.

Therefore, we combined the two matrices into a single plastid data set. Sampling was uneven due to eight missing *trnD-trnT* sequences and one missing *trnT-trnF* sequence, representing 13.2% of the data. Parsimony analysis yielded 9310 MPT of 475 steps (CI = 0.939; RI = 0.801; RC = 0.752); the strict consensus of those trees is presented in Figure 2. Combining the plastid data sets resulted in greater support for the clades present in the separate plastid analyses, with additional groupings not present in the ITS or separate plastid analyses. A clade containing *Camplocarpus mauritianus* (Lam.) Decne., *Cryptostegia madagascariensis* Bojer, *Ischnolepis graminifolia* (Constantin & Gallaud) Klack., *Pentopetia grevei* (Baill.) Venter, and *P. longipetala* Klack., representing four of five Malagasy genera (*Baroniella* Constantin & Gallaud is absent from this data set) is supported (BS 82). Four genera are monophyletic: *Cryptolepis* (BS 79), *Pentopetia* (BS 68), *Periploca* (BS 100; *P. nigrescens* absent from plastid data set), and *Raphionacme* (BS 97; *R. lobulata* absent from plastid data set). *Ectadium latifolium* N. E. Br. is weakly supported as sister to *Periploca* (64% BS); this relationship was not supported by the ITS analysis. *Schlechterella abyssinica* (Chiov.) Venter & R. L. Verh. is sister to *Raphionacme* (BS 72). The Asian pollinial clade identified in the ITS analysis is represented in the combined plastid analysis by four taxa, which again form a clade (BS 81). Within this clade, *Finlaysonia* is polyphyletic, in agreement with the results of the ITS analysis.

TOTAL EVIDENCE

Visual inspection of the separate analyses of the nuclear and plastid data sets revealed no strongly supported contradictory clades, and a partition homogeneity test showed that the plastid and ITS data sets are concordant ($P = 0.28$); therefore, we combined the data sets. Sampling was uneven due to 24 missing *trnD-trnT* sequences and 17 missing *trnT-trnF* sequences, representing 34.6% of the data. Parsimony analysis of the total evidence data set resulted in 142 MPT of 1706 steps (CI = 0.601; RI = 0.620; RC = 0.373); one of the 142 shortest trees is presented in Figure 3. This analysis yielded a strict consensus tree similar in topology to the ITS consensus tree, with better resolution in portions of the tree and better support for most nodes. As in the ITS analysis, *Phyllanthera grayi* is supported as sister to the rest of the subfamily, a relationship not seen in the plastid analyses. The Asian pollinial clade (BS 71), the grooved translator clade (BS 94), the *Cryptolepis*–*Omphalogonus calophyllus*–*Periploca nigrescens* clade (BS 97), and the *Periploca laevigata* Ait. through *P.*

Table 1. Voucher information for plant material used in this study. Sequences previously published by Meve and Liede (2004) are indicated with an asterisk. Dashes indicate missing sequences. Two GenBank numbers are given for the *trnT-F* region of *Sacleuxia newii*; the first number represents *trnL-F* and the second (last two digits given) represents *trnT-L*.

Taxon	Origin	Voucher	GenBank accession numbers		
			<i>trnT-F</i>	<i>trnD-T</i>	ITS
OUTGROUP					
<i>Forsteronia leptocarpa</i> A. DC.	Brazil, Ubatuba	<i>Simões & Singer 1037</i> (UEC)	DQ916868	—	DQ916831
<i>Rhabdadenia biflora</i> Müll. Arg.	U.S.A., Florida	<i>Zona 616</i> (FTG)	DQ916869	DQ916903	DQ916832
PERIPLOCOIDEAE					
<i>Atherandra acuminata</i> Decne.	Thailand, Surat	<i>Put. 4139</i> (P)	—	—	DQ916833
<i>Baroniella camptocarpoides</i> Costantin & Gallaud	Madagascar, Toliara	<i>Phillipson et al. 3956</i> (MO)	—	—	DQ916834
<i>Baseonema gregorii</i> Schltr. & Rendle	Tanzania	<i>Cox & Abdallah 2028</i> (K)	—	—	DQ916835
<i>Batesanthus purpureus</i> N. E. Br.	Zaire	<i>Hart 620</i> (MO)	—	—	DQ916836
<i>Buckollia volubilis</i> (Schltr.) Venter & R. L. Verh.	Ethiopia, Sidamo	<i>Hangen 2041</i> (K)	—	—	DQ916837
<i>Camptocarpus mauritianus</i> (Lam.) Decne.	Madagascar, Toliara	<i>Petignat s.n.</i> (UBT)	*	DQ916887	*
<i>Cryptolepis buchananii</i> Roem. & Schult.	Pakistan, Islamabad	<i>Sultan s.n.</i> (UBT)	DQ916870	DQ916888	DQ916838
<i>Cryptolepis capensis</i> Schltr.	South Africa	<i>Abbott 7761</i> (Z)	DQ916871	DQ916889	DQ916839
<i>Cryptolepis delagoensis</i> Schltr.	South Africa, KwaZulu-Natal	<i>Bruyns 9357</i> (BOL)	—	—	DQ916840
<i>Cryptolepis hypoglauca</i> K. Schum. ex Engl.	Tanzania, Masasi Distr.	<i>Bidgood et al. 2086</i> (K)	—	—	DQ916841
<i>Cryptolepis oblongifolia</i> (Meisn.) Schltr.	South Africa, Transvaal	<i>Bruyns 9367</i> (E)	DQ916872	DQ916890	DQ916842
<i>Cryptostegia madagascariensis</i> Bojer	U.S.A., Florida, cult.	<i>Ionta 418</i> (FLAS)	DQ916873	DQ916891	DQ916843
<i>Cryptostegia madagascariensis</i> Bojer	U.S.A., Florida, cult.	<i>Ionta 538</i> (FLAS)	—	—	DQ916844
<i>Decalepis arayalpathra</i> (J. Joseph & V. Chandras.) Venter	India, Cirzar Hills, 1989	<i>Rajasekharan s.n.</i> (K)	—	—	DQ916845
<i>Ectadium latifolium</i> N. E. Br.	Namibia	<i>Albers 2413</i> (UBT)	DQ916874	DQ916892	DQ916846
<i>Epistemma rupestre</i> H. Huber	Cameroon, Rocher Ako'okas	<i>deWilde 7462</i> (MO)	—	—	DQ916847
<i>Finlaysonia insularum</i> (King & Gamble) Venter	Thailand	<i>Middleton 1164</i> (A)	DQ916875	DQ916893	DQ916848
<i>Finlaysonia lanuginosa</i> (Ridl.) Venter	U.S.A., Cornell Univ., cult.	<i>Livshultz 03-41</i> (BH)	DQ916876	DQ916894	DQ916849
<i>Gymnanthera oblonga</i> (Burm. f.) P. S. Green	Australia, Northern Territory	<i>Forster 6133</i> (K)	—	—	DQ916850
<i>Hemidesmus indicus</i> (L.) R. Br. ex Schult.	India	<i>Civeyrel 1008</i> (TL)	DQ916877	DQ916895	DQ916851
<i>Ischnolepis graminifolia</i> (Costantin & Gallaud) Klack.	Madagascar, Ambinanitelo	<i>Röösli s.n.</i> (UBT)	*	—	*
<i>Mondia whiteii</i> (Hook. f.) Skeels	South Africa, Univ. Natal Pietermaritzberg, cult.	<i>ex hort. Ollerton s.n.</i>	DQ916878	DQ916896	DQ916852

Table 1. Continued.

Taxon	Origin	Voucher	GenBank accession numbers		
			<i>trnT-F</i>	<i>trnD-T</i>	ITS
<i>Myriopteron paniculatum</i> Griff.	s. loc.	<i>Shuncheng 9801135</i> (MO)	—	—	DQ916853
<i>Omphalogonus calophyllus</i> Baill.	Tanzania, Muheza District	<i>Mwangoka et al. 1241</i> (MO)	—	—	DQ916854
<i>Pentopetia grevei</i> (Baill.) Venter	Madagascar, Ambinanitelo	<i>Mangelsdorff 516</i> (UBT)	*	DQ916897	*
<i>Pentopetia longipetala</i> Klack.	Madagascar, Ambalabao	<i>Allorge 2422</i> (P)	DQ916879	—	DQ916855
<i>Periploca calophylla</i> (Wight) Falc.	China, Sichuan Prov.	<i>Liu Zheng Yu 15548</i> (GH)	DQ916880	—	DQ916856
<i>Periploca graeca</i> L.	Switzerland, Bot. Gard. Zurich, cult.	<i>Endress s.n.</i> (Z)	DQ916881	DQ916898	DQ916857
<i>Periploca laevigata</i> Ait.	Spain, Canary Islands	<i>Fernandez s.n.</i> (Z)	DQ916882	DQ916899	DQ916858
<i>Periploca nigrescens</i> Afzel.	Ghana	<i>Merello et al. 1354</i> (MO)	—	—	DQ916859
<i>Periploca visciformis</i> (Vatke) K. Schum.	Socotra, Wadi Ayhaft	<i>Mies 1444</i> (UBT)	*	—	*
<i>Petopentia natalensis</i> (Schltr.) Bullock	s. loc.	<i>ex hort. Kiel.</i> (UBT)	*	DQ916900	*
<i>Phyllanthera grayi</i> (P. I. Forst.) Venter	Australia	<i>Forster 24232</i> (BRI)	DQ916883	DQ916901	DQ916860
<i>Raphionacme flanaganii</i> Schltr.	South Africa, Pietermaritzburg	<i>Albers 1577</i> (MSUN)	*	—	*
<i>Raphionacme galpinii</i> Schltr.	South Africa	<i>Abbott s.n.</i> (Z)	DQ916884	DQ916902	DQ916861
<i>Raphionacme lobulata</i> Venter & R. L. Verh.	South Africa, Eastern Cape	<i>Dold 4461</i> (GRA)	—	—	DQ916862
<i>Raphionacme madiensis</i> S. Moore	Tanzania, Dodoma	<i>Specks 682</i> (UBT)	*	—	*
<i>Sacleuxia newii</i> (Benth.) Bullock	Tanzania, Kilosa	<i>Bruyns 8653</i> (K)	DQ916909/10	—	DQ916863
<i>Schlechterella abyssinica</i> (Chiov.) Venter & R. L. Verh.	Kenya, Furole	<i>Newton 4555</i> (UBT)	*	DQ916904	*
<i>Stomatostemma monteiroae</i> N. E. Br.	s. loc.	<i>IPPS 1427</i> (UBT)	*	DQ916905	*
<i>Stomatostemma pendulina</i> Venter & D. V. Field	Mozambique, Ribaue	<i>Bruyns 7716</i> (K)	—	—	DQ916864
<i>Streptocaulon juvenas</i> (Lour.) Merr.	Laos, Sii Phan Don, Kong Distr.	<i>Middleton 276</i> (A)	—	DQ916906	DQ916865
<i>Tacazzea apiculata</i> Oliver	Zambia, Luapula District	<i>Renvoize 5760</i> (MO)	DQ916885	DQ916907	DQ916866
<i>Zygostelma benthamii</i> Baill.	Thailand, Phetchaburi	<i>Middleton 849</i> (A)	DQ916886	DQ916908	DQ916867

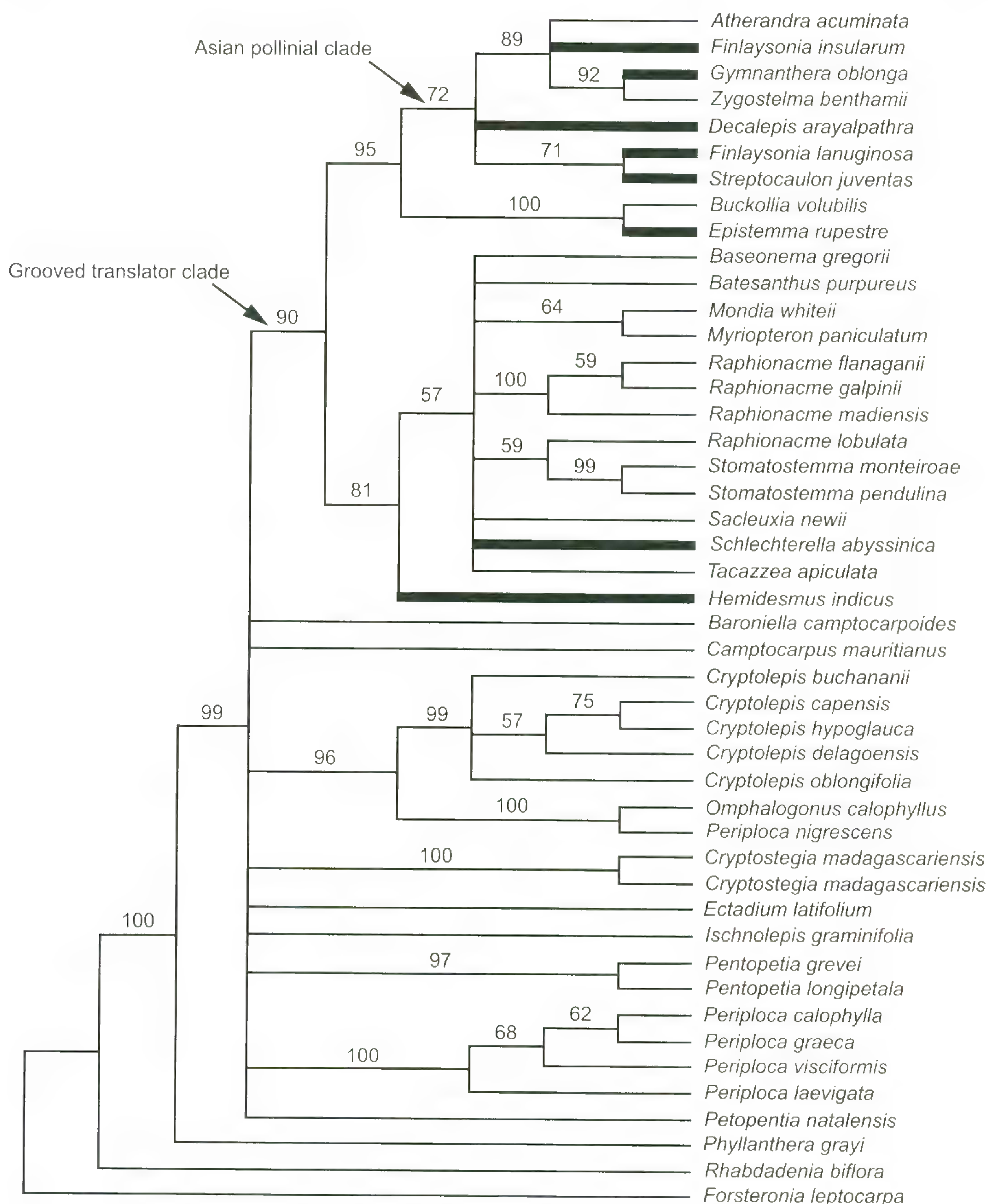


Figure 1. Strict consensus of 93 equally parsimonious trees (tree length [L] = 1223, CI = 0.474, RI = 0.609, RC = 0.289) resulting from parsimony analysis of ITS sequence data. Branches in bold indicate pollinal taxa. Numbers above the branches indicate bootstrap values.

calophylla (Wight) Falc. clade (BS 100), all present in the ITS analysis, are supported. *Finlaysonia* is polyphyletic; *F. lanuginosa* is sister to *Streptocaulon juvenas* with greater support than in individual analyses (BS 75). Relationships among those taxa outside of the grooved translator clade are not well resolved. Representatives of two of five Malagasy genera (*Camptocarpus* Decne. and *Cryptostegia* R. Br.)

appear together in 100% of the MPT, but there is no statistical support for this relationship.

MORPHOLOGY

Morphological assessments were made based on a combination of previously published data (Verhoeven & Venter, 1988, 1989, 1994a, b, 1997, 1998a, b,

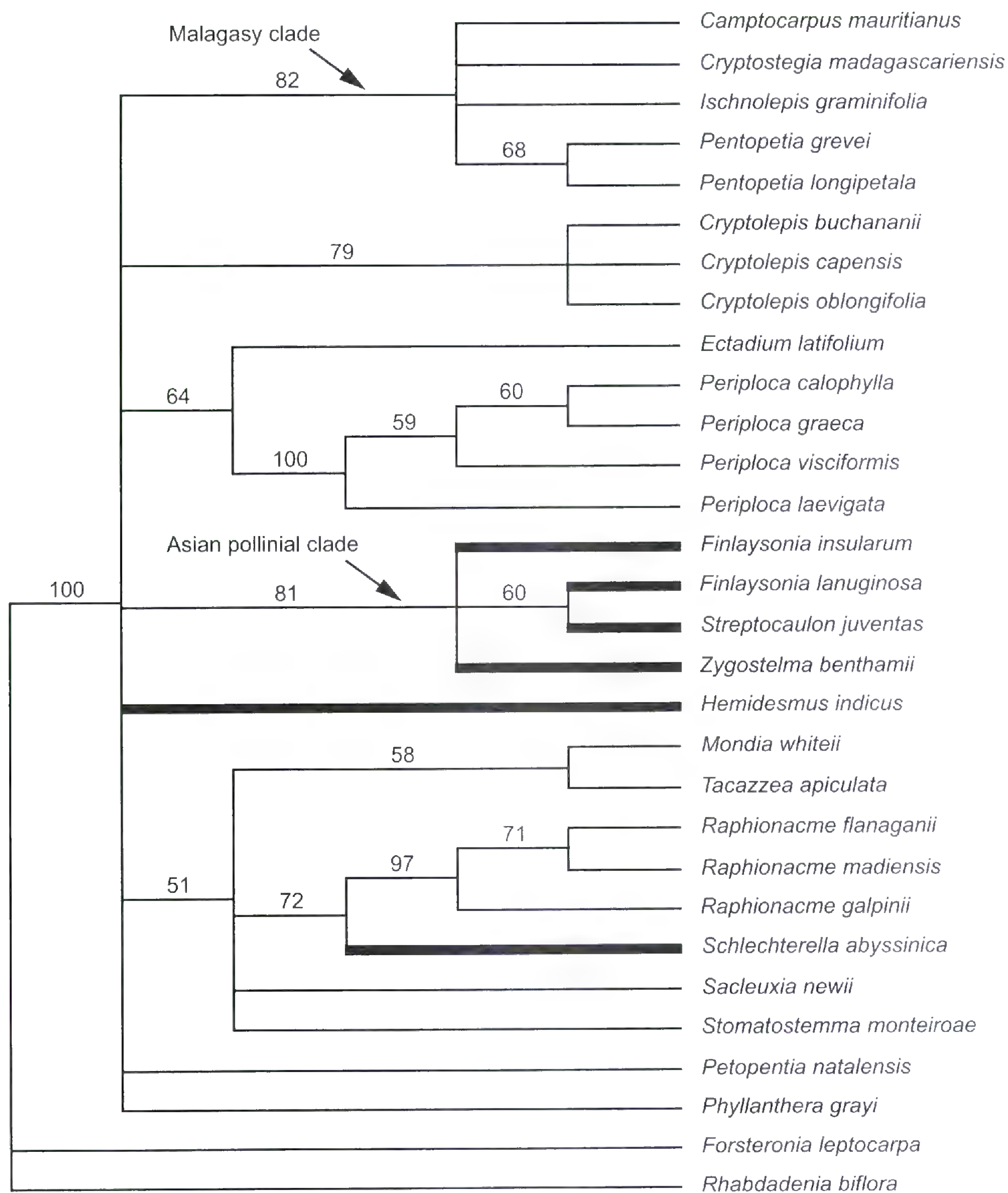


Figure 2. Strict consensus of 9310 equally parsimonious trees ($L = 475$, $CI = 0.939$, $RI = 0.801$, $RC = 0.752$) resulting from parsimony analysis of combined plastid data. Branches in bold indicate pollinial taxa. Numbers above the branches indicate bootstrap values.

2001, 2002; Verhoeven et al., 1989; Nilsson et al., 1993) and our own observations. We examined spirit-preserved flowers and/or herbarium specimens of all taxa included in this study, with a particular emphasis on pollen and pollen translators. The evolution of pollinia was analyzed by mapping the presence or absence of this character (using parsimony) onto one of the shortest trees from our total evidence analysis in MacClade 4.0 (Maddison & Maddison, 1999). Our investigation into the pollen morphology of Periplocoideae is preliminary and only summarized in this paper.

In a majority of taxa, we observed pollen tetrads (not gathered into pollinia) that were round to slightly oblong ($L/W \leq 1.5$; Fig. 4C), with pollen grains arranged tetrahedrally, decussately, and/or rhomboidally. The exceptions were: (1) *Periploca graeca* L. (non-pollinial), in which we observed oblong ($L/W = 1.5\text{--}2.0$), decussate, and rhomboidal tetrads (Fig. 4E); (2) *Petopentia natalensis* (non-pollinial), with a mix of rhomboidal, linear, and T-shaped tetrad shapes ($L/W = 1.5\text{--}4.0$); (3) members of the Asian pollinial clade (most pollinial) with elongate ($L/W = 2.0\text{--}6.0$; Fig. 4G, H) T-shaped, rhomboidal, or linear tetrads;

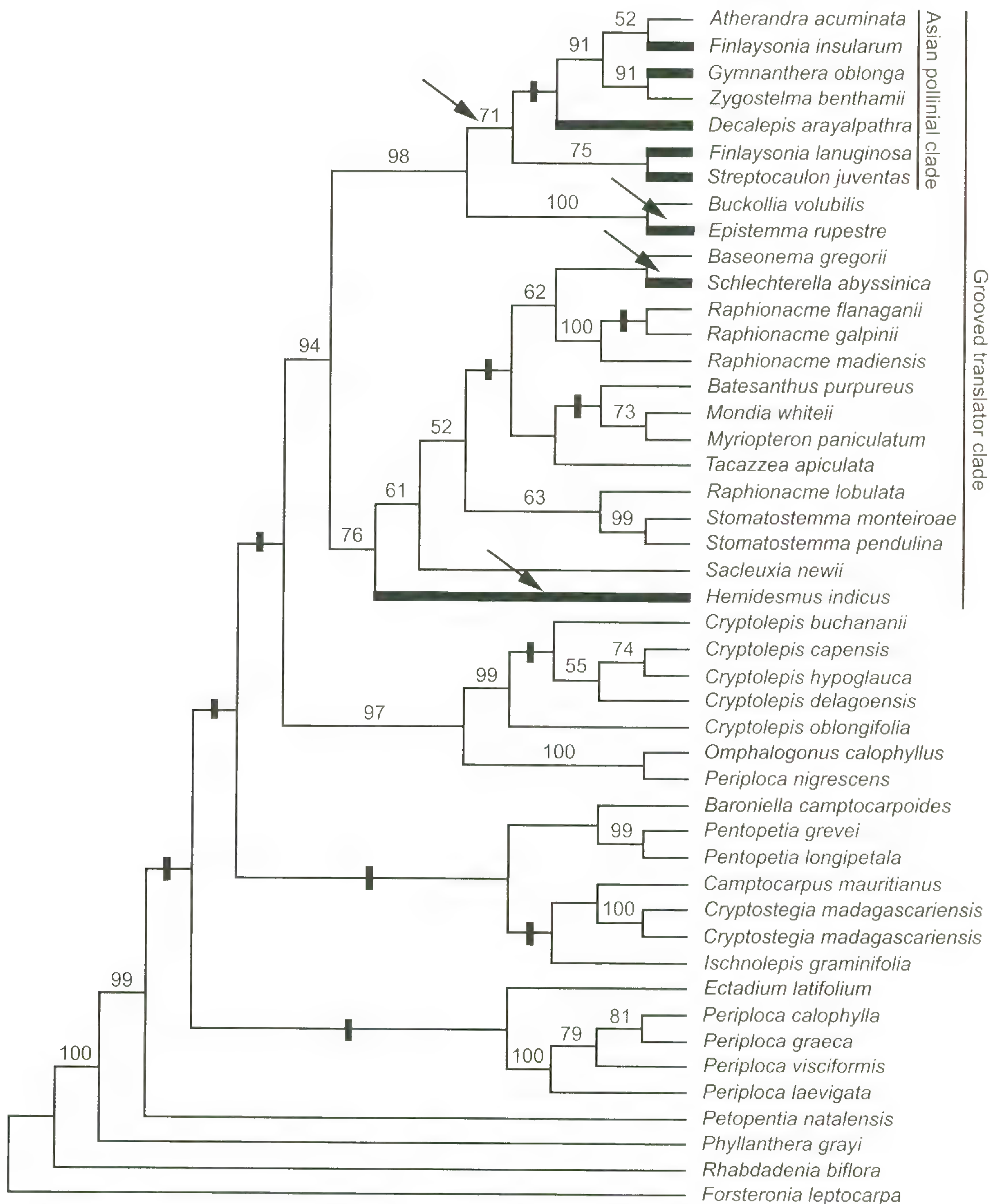


Figure 3. One of 142 equally parsimonious trees ($L = 1706$, $CI = 0.601$, $RI = 0.620$, $RC = 0.373$) resulting from parsimony analysis of ITS and plastid data combined. Branches in bold indicate pollinial taxa. Numbers above the branches indicate bootstrap values. Putative origins of pollinia using DELTRAN optimization are marked with arrows. Bars indicate branches that collapse in the strict consensus of all shortest trees.

(4) *Epistemma rupestre* (pollinial), with a mix of rounded and elongate rhomboidal tetrads ($L/W = 1-2.5$); (5) *Hemidesmus indicus* (L.) R. Br. ex Schult. (pollinial), with a mix of rhomboidal, linear, and T-shaped tetrads ($L/W = 1.5-3.0$); and (6) *Schlechterella abyssinica* (pollinial), with oblong, decussate tetrads ($L/W = \text{ca. } 2.0$). Tetrads were arranged

randomly on the translator spoon with the following exceptions: in both pollinial and non-pollinial members of the Asian pollinial clade, tetrads were aligned more or less parallel to one another (in a fan-like fashion when the tetrads were T-shaped) and roughly perpendicular to the translator spoon, and in the pollinial taxon *Schlechterella abyssinica*, tetrads were

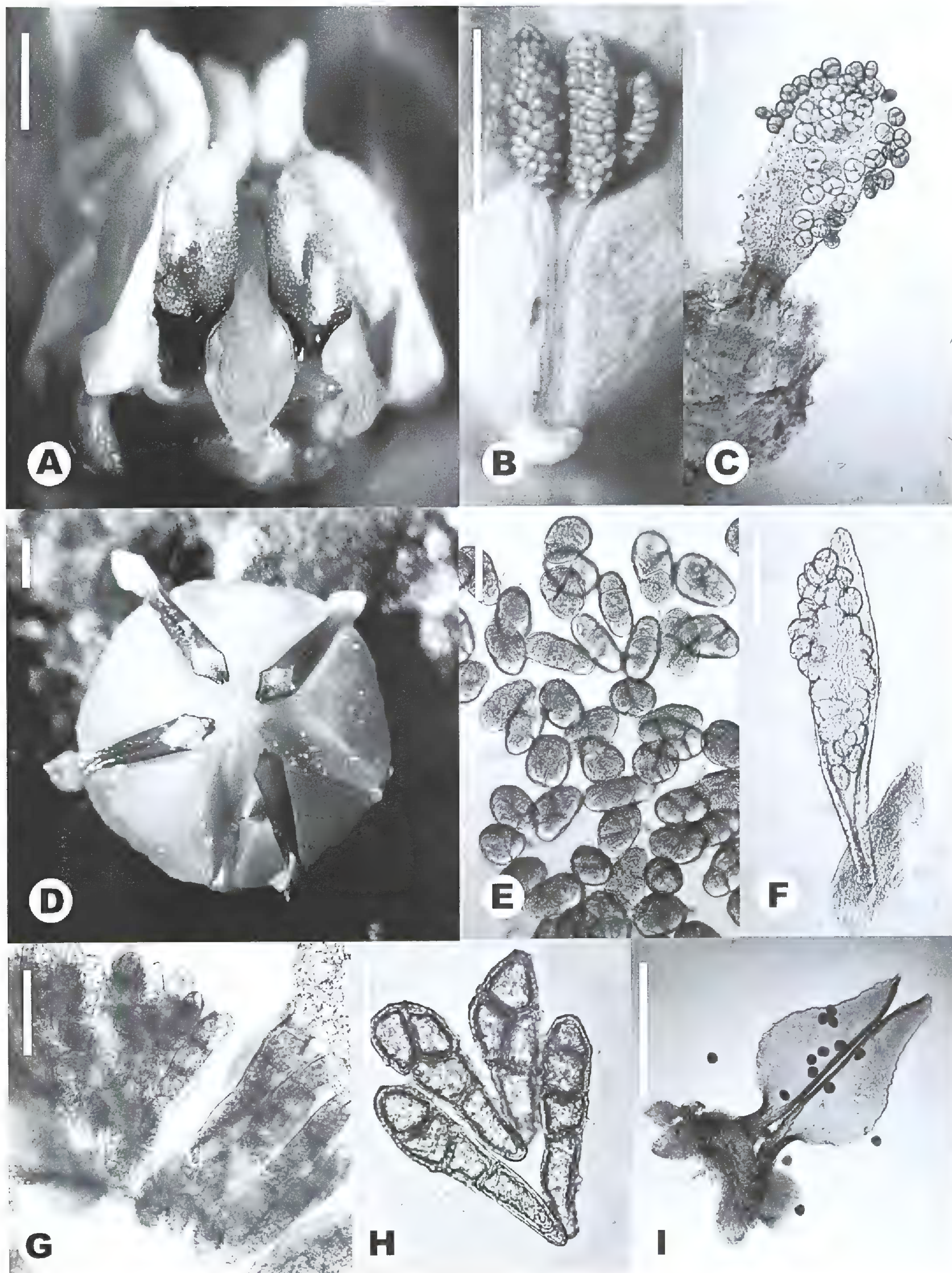


Figure 4. Translators and pollen of Periplocoideae. —A. *Cryptostegia madagascariensis*. Gynostegium with two anthers removed to show translators lying on stylar head. *Ex hort.* (FTG). —B. *Gymnanthera oblonga*. Translator with pollinia in position at anthesis on stylar head. *Forster 6133* (K). —C. *Ectadium latifolium*. Simple translator spoon and rounded tetrads. *Albers 2413* (UBT). —D. *Mondia whiteii*. Stylar head from above, with translators. Note deep longitudinal groove in translator spoon and divided apex. *Ex hort.* *Ollerton s.n.* —E. *Periploca graeca*. Pollen tetrads. *Endress s.n.* (Z). —F. *Cryptolepis buehnanii*. Simple translator spoon with rounded tetrads. *Grierson & Long 2060* (A). —G. *Decalepis hamiltonii*. Elongate, linear tetrads forming pollinia. Note parallel alignment of tetrads. *Nicholson, Saldanha & Ramamoorthy HFP163* (MO). —H. *Finlaysonia insularum*. Four elongate, linear, and T-shaped tetrads removed from pollinium. *Chin 1743* (K). —I. *Stomatostemma monteiroae*. Translator. Note deep groove in translator spoon and rounded tetrads. *Albers & Mene 518* (UBT). Scale bars: A, D = 1 mm; B, I = 500 µm; C, F = 100 µm; E, G, H = 50 µm.

mostly randomly aligned, but at least some were aligned parallel to one another. Tetrad alignment in the pollinial taxa *Epistemma rupestre* and *Hemidesmus indicus* appeared to be random.

DISCUSSION

PHYLLANTHERA: SISTER TO THE REST OF PERIPLOCOIDEAE?

Phyllanthera grayi is supported as sister to the rest of Periplocoideae in the ITS and total evidence analyses. As currently circumscribed, *Phyllanthera* Blume comprises eight species of Southeast Asian and Australian forest climbers. Forster (1993) placed *Phyllanthera* (then comprised of two species) into synonymy with *Cryptolepis*, recognizing these two taxa plus four *Cryptolepis* species as the subgenus *Phyllanthera*. Venter and Verhoeven (2001) later reinstated the genus *Phyllanthera*, uniting *Cryptolepis* subgenus *Phyllanthera* with the monotypic genus *Pentanura* Blume on the basis of similar floral structures, including a lack of alternipetalous corona lobes, prominent anther apices, and staminal feet swollen and fused around the style.

Phyllanthera grayi (originally described under *Cryptolepis* subgenus *Phyllanthera*) is the sole representative of the genus in our analyses. Its placement at the base of the subfamily comes as a surprise. Endress and Bruyns (2000) described a morphological transformation series in flowers of Periplocoideae leading from (1) *Gymnanthera*-type flowers, with staminal feet running down a long lower corolla tube made up of congenitally fused corolla, stamens, and nectaries (Nishino, 1982; Venter & Verhoeven, 2001), similar to flowers of Rauvolfioideae and Apocynoideae; to taxa in which (2) the lower corolla tube is much shorter, with incompletely fused staminal feet forming a ring around the top of the ovary; to a condition where (3) the staminal feet are completely fused around the style (e.g., *Hemidesmus*); approaching in form the (4) "staminal tube" of Secamonoideae/Asclepiadoideae. Under this scenario, one would expect the early-diverging members of Periplocoideae to have a long corolla tube as in the first condition described above; however, *Phyllanthera*, with staminal feet fused incompletely into a ring around the style, exemplifies the second condition.

Venter and Verhoeven (1997) placed *Phyllanthera* in the tribe Cryptolepideae. The presence of a distinct corolla tube in members of Cryptolepideae led them to hypothesize that this tribe was more advanced than, and perhaps derived from, the tribe Periploceae. They later abandoned this tribal classification based on the results of their own phylogenetic analysis of morphological characters (Venter & Verhoeven, 2001). It is

of interest, however, that in their morphology-based phylogenetic analysis *Phyllanthera* again appeared in a derived position (within the "Atherandra clade"), in support of the views of Endress and Bruyns (2000), and in conflict with its placement in our DNA-based trees.

At this time, our morphological investigations are still in a preliminary stage. Given the absence of clear morphological characters supportive of *Phyllanthera* in this position, coupled with a lack of support for this relationship in the plastid analyses, we consider the phylogenetic placement of *Phyllanthera* as sister to the remaining genera of Periplocoideae to be an interesting, but still tentative, hypothesis.

A GROOVED TRANSLATOR CLADE

A clade containing roughly half of the species sampled (23 of 43 ingroup taxa) appears in both the ITS (Fig. 1, BS 90) and total evidence analyses (Fig. 3, BS 94). This clade, comprising African and Asian taxa, includes the eight pollinial exemplars. Most taxa in this clade have pollen translators with a prominent groove (corresponding to a ridge in the stylar head where the translator is formed) running transversely along the spoon (Fig. 4D, I). Often there is a notch at the top of the spoon, which may extend longitudinally down the spoon, partially dividing it into two halves. Exceptions are the genus *Raphionacme*, in which the groove is absent or shallow, and *Baseonema gregorii* Schltr. & Rendle, *Sacleuxia newii* (Benth.) Bullock, and *Schlechterella abyssinica*, whose translators have a shallow groove. These likely represent reversals.

Taxa outside of this grooved translator clade bear translators with flattened spoons and rounded apices (e.g., *Ectadium latifolium*; Fig. 4C); in some cases (e.g., *Petopentia natalensis*) the central axis of the spoon is slightly thinner. We note that the Malagasy taxa sampled in this study (representing all five Malagasy genera) fall outside of the grooved translator clade. Arekal and Ramakrishna (1980: 430) observed that translator spoons in *Decalepis* and *Hemidesmus* (both within the grooved translator clade) had "a median partition wall, dividing it into two parts, each of which receives pollen tetrads of the nearest anther lobe." They interpreted these translators as more specialized than the un-grooved translators of *Cryptolepis buchananii* (Fig. 4F) and *Cryptostegia madagascariensis* (Fig. 4A), neither of which appeared in the grooved translator clade in our analyses. Safwat (1962: 113) noted that "the genus *Cryptolepis* is considered to be rather primitive because of the poorly developed translators, a fact which led R. Brown to include it within the Apocynaceae."

AN ASIAN POLLINIAL CLADE

There is moderate support for a clade of seven Asian species (five of which bear pollinia) in the ITS (Fig. 1, BS 72), plastid (four taxa in the plastid data set, Fig. 2, BS 81), and total evidence analyses (Fig. 3, BS 71). In the ITS and total evidence analyses, this clade appears within the grooved translator clade. Members of the Asian pollinial clade appear in all three tribes of Venter and Verhoeven (1997) and did not form a clade in their morphology-based cladistic analysis (2001). This conclusion of Verhoeven and Venter (1997, 2001) is understandable; these are, morphologically, seemingly disparate taxa with little uniting them beyond the presence of pollinia in most. *Gymnanthera oblonga*, for example, has flowers up to 17 mm long, a long corolla tube, and squat, transverse-triangular coronas (Forster, 1991). In contrast, *Zygostelma benthamii*, also in the Asian pollinial clade (sister to *G. oblonga*, Fig. 3, BS 91), has flowers ca. 6 mm long, a short corolla tube, and unique, curiously shaped coronas that resemble a pair of ram's curved horns. The Asian pollinial clade exemplifies the wide range of morphological variation found in closely related taxa throughout the subfamily; this variability, in part, explains the large number of monotypic and ditypic genera that have been recognized in Periplocoideae, as well as the difficulty in assessing relationships on the basis of morphology alone.

Pollen and translators offer clues to intergeneric relationships in Periplocoideae. In most taxa, pollen tetrads are round or short-oblong and arranged randomly on the translator spoon (Fig. 4A, C, I). In contrast, we observed that both pollinia and tetrad-bearing members of the Asian pollinial clade have elongate pollen tetrads that are aligned roughly parallel to one another (sometimes the tetrads are wider at the distal end; this results in a fanlike arrangement) and more or less perpendicular to the translator spoon (Fig. 4B, G, H). Tetrad shape apparently does show some homoplasy in Periplocoideae. We observed a mix of tetrad shapes including elongate forms in *Periploca graeca* and *Pentopentia natalensis* and in the three pollinial taxa appearing outside of the Asian pollinial clade in our analyses: *Epistemma rupestre* has rounded to oblong tetrads, *Hemidesmus indicus* has a mix of shapes, and *Schlechterella abyssinica* has oblong, decussate tetrads. Thus, with a few exceptions, there is an intriguing trend from more or less rounded tetrads in non-pollinial Periplocoideae toward the elongation of pollen tetrads in the pollinial taxa and in the tetrad-bearing members of the Asian pollinial clade.

The genus *Atherandra* Decne. has been documented as having pollen arranged in tetrads (Venter &

Verhoeven, 2001). Prior to including *Atherandra acuminata* in our phylogenetic analyses, we noted that in this species, translator shape and the shape and arrangement of pollen tetrads is similar to that of species in the Asian pollinial clade: the tetrads are elongate, aligned parallel to one another, and roughly perpendicular to the translator spoon. Based solely on this, we speculated that *A. acuminata* would group within the Asian pollinial clade, despite having tetrads rather than pollinia. Indeed, when included in our analysis, *A. acuminata* grouped with the Asian pollinial taxa, with good support (Fig. 3, BS 71).

A MALAGASY CLADE?

Five Periplocoideae genera are endemic to Madagascar and the Mascarene Islands: *Baroniella*, *Camptocarpus*, *Cryptostegia*, *Ischnolepis*, and *Pentopetia*. The relationship of these Malagasy genera to each other and to other Periplocoideae is poorly resolved in the ITS and combined analyses, but in the combined plastid analysis (*Baroniella* is missing from the plastid data sets) they form a well-supported clade (Fig. 2, BS 82). This is in accordance with the findings of Meve and Liede (2004); their analyses of plastid data supported a Malagasy clade (*Camptocarpus*–*Ischnolepis*–*Pentopetia*), a relationship not recovered from their analyses of ITS and total evidence data sets. The hypothesis of a Malagasy clade is compelling; however, at this time there is insufficient support for this grouping.

MONOPHYLY OF GENERA AND THE GENUS *PERIPLOCA*

Our analyses support the monophyly of *Stomatostemma*, *Pentopetia*, and *Cryptolepis* (Figs. 1, 3). Both species of *Stomatostemma* were sampled; *Pentopetia* and *Cryptolepis*, however, are fairly large genera (21 and 27 species, respectively); thus, further taxon sampling is warranted in order to confirm our results. *Periploca*, the type genus of the subfamily, occurs in a variety of habitats in Asia and Africa and extends into Europe. The genus exhibits a wide range of growth types, from forest climber to scrambling or erect shrub to geophytic herb (Browicz, 1966; Venter, 1997). Venter (1997) delimited two sections in *Periploca* based on the presence (section *Periploca*, further divided into three subgroups, or series) or absence (section *Monocoronata*) of basally tri-segmented corona lobes. In our analyses, which included exemplars from all four subgroups, representatives of section *Periploca* (*P. calophylla*, *P. graeca*, *P. visciformis*, and *P. laevigata*) form a well-supported clade. *Periploca nigrescens*, in contrast (representing section *Monocoronata*, the other main group delimited

by Venter), is well supported as sister to *Omphalogonus calophyllus* (Fig. 3, BS 100); together, these two taxa are sister to *Cryptolepis*.

The genus *Periploca* is quite distinctive: nine of 14 species have a dark fleshy center on the adaxial corolla lobes, often surrounded by hairs or with a white papillose spot in the center. These corolla features, unique to *Periploca* and perhaps synapomorphic, are not present in *P. nigrescens* or other members of section *Monocoronata*. Venter and Verhoeven (1997) hypothesized that section *Monocoronata* represented the least modified of *Periploca* species and further speculated that *P. nigrescens* was most likely not closely related to other members of that section. Inclusion of additional exemplars from *Periploca* section *Monocoronata* is planned in order to further test the monophyly of the genus.

OMPHALOGONUS CALOPHYLLUS-PERIPLOCA NIGRESCENS

Periploca nigrescens and *Omphalogonus calophyllus* form a well-supported clade in the ITS and total evidence analyses (Figs. 1, 3, BS 100). It is difficult to distinguish between these two species based solely on vegetative characters; both form large lianas with leaves of similar shape, texture, and appearance, and the plants are black when dried, a condition not present in other Periplocoideae. They are so similar in vegetative form that Bullock (1961) combined *Periploca nigrescens*-*Parquetina gabonica* Baill.-*Omphalogonus calophyllus* into a single species, *Parquetina nigrescens* (Afzel.) Bullock. Floral morphology in these taxa does, however, differ: the flowers of *P. nigrescens* are rotate, the gynostegium is exserted, and the anthers are large, while in *Omphalogonus*, flowers are campanulate with an enclosed gynostegium and less-conspicuous anthers. Recognizing these differences in floral morphology, Venter and Verhoeven (1996) later reinstated *O. calophyllus* and *P. nigrescens* as distinct species in two different genera, and in 1997 even placed them in separate tribes. Our findings that *P. nigrescens* and *O. calophyllus* are well supported as sister taxa illustrate the importance of considering both floral and vegetative features when assessing relationships within this complex group.

A POLYPHYLETIC FINLAYSONIA

Venter and Verhoeven (2001) placed several monotypic and ditypic Asian genera into synonymy under the previously monotypic *Finlaysonia* due to similarities in floral form (the gynostegium exposed from a rotate or saucer-shaped corolla) and because all have pollen fused into pollinia. We included in our study two *Finlaysonia* species: *F. insularum* (=Gym-

nanthera insularum King & Gamble, later *Meladerma insularum* (King & Gamble) Kerr.) and *F. lanuginosa* (= *Gongylosperma lanuginosum* Ridley). We were unable to obtain suitable material for DNA sequencing of *F. obovata* Wall., the generic type. Our analyses did not support the monophyly of *Finlaysonia* s.l.: in the ITS and combined analyses, *F. insularum* appears in a clade with *Atherandra acuminata*-*Gymnanthera oblonga*-*Zygostelma benthamii* (Figs. 1, 3), while *F. lanuginosa* pairs with *Streptocaulon juvenas*. The plastid data set also strongly supports the relationship between *F. lanuginosa* and *S. juvenas* (Fig. 2). *Finlaysonia lanuginosa* has a densely pubescent abaxial leaf surface similar to that seen in *Streptocaulon* species, in contrast to the glabrous condition observed in other *Finlaysonia* species examined. *Finlaysonia* as currently circumscribed is an amalgamation of six smaller genera recently placed in synonymy (Venter & Verhoeven, 2001). We hope to include as many of these species as possible in future analyses of both molecular and morphological data.

RAPHIONACME LOBULATA RENDERS RAPHIONACME NON-MONOPHYLETIC

In a cladistic analysis of molecular data, which included 17 species in nine genera of Periplocoideae, Meve and Liede (2004) found good support for the monophyly of *Raphionacme*, a genus of 37 species. The inclusion of *R. lobulata* in our analyses rendered *Raphionacme* non-monophyletic; *R. lobulata* is not allied with other *Raphionacme* exemplars and is instead supported as sister to *Stomatostemma* (Figs. 1, 3). Venter and Verhoeven (1988) originally placed *R. lobulata* in the genus *Raphionacme* based on similarities in floral features such as corolla structure and the position of stamens and corona lobes on the corolla tube. They noted pollen grains with eight pores in *R. lobulata*. Most Periplocoideae have four to six pores per pollen grain; *Raphionacme*, *Schlechterella*, and *Baseonema* Schltr. & Rendle have eight to 16. Venter et al. (2006) later noted that vegetatively *R. lobulata* more closely resembles *Stomatostemma* (similar leaves and habit, and similar strings of spherical tubers on their roots) and *Batesanthus* N. E. Br. (similar fleshy interpetiolar stipules) than *Raphionacme*, and a re-investigation of pollen in this taxon showed four to six pores per grain. Our results, which indicate a close relationship between *R. lobulata* and *Stomatostemma*, are in accordance with these observations. Here again we see that vegetative and pollen characters can be more informative than gross floral morphology in assessing relationships in Periplocoideae.

EVIDENCE FOR MULTIPLE ORIGINS OF POLLINIA

We mapped the presence of pollinia onto one of the shortest trees from the total evidence analysis; our results indicate either three or four separate origins of pollinia in Periplocoideae, each of which require four evolutionary changes. Under DELTRAN optimization (Fig. 3), pollinia are hypothesized to have originated four times: once in the common ancestor of the Asian pollinial clade, two more times (separately) in the African genera *Epistemma* and *Schlechterella*, and a fourth time in *Hemidesmus*, a monotypic genus from India. Under ACCTRAN optimization, pollinia are hypothesized to have evolved three times: in the common ancestor of the Asian pollinial–*Epistemma*–*Buckollia* Venter & R. L. Verh. clade, again in *Schlechterella*, and a third time in *Hemidesmus*. Thus, it is equally parsimonious to view the pollinia of *Epistemma* as homologous with those of the Asian pollinial clade (in which case *Buckollia* represents a reversal to tetrads) or as representing yet a fourth separate origin of pollinia in the subfamily. These results support the hypothesis of Fishbein (2001) that pollinia evolved more than once in Periplocoideae, and more specifically, the hypotheses of Venter and Verhoeven that pollinia in Asian and African Periplocoideae evolved polyphyletically (1997) and independently (2001).

Our preliminary observations suggest that pollinial form varies in a manner consistent with phylogenetic pattern and is suggestive of multiple origins of pollinia within the subfamily. The pollinia of *Schlechterella* are very different from those found in other pollinial taxa. The most striking difference can easily be seen in SEM images: in all other species with pollinia, the distal tetrad walls are inaperturate or vestigially porate (Verhoeven & Venter, 2001), while in *Schlechterella* (Verhoeven & Venter, 1998b), the pollen tetrads have pores on both proximal and distal walls. The proximal walls of a pollinium are defined as walls between adjacent tetrads; distal walls are on the outside of the pollinium (Verhoeven & Venter, 1994b, 1998a).

We observed elongate pollen tetrads aligned parallel to one another and relatively perpendicular to the translator spoon in members of the Asian pollinial clade (pollinial and tetrad-bearing taxa). This is in contrast to the randomly aligned tetrads of *Schlechterella* and non-pollinial taxa. We were unable to determine the orientation of tetrads to the translator spoons in *Hemidesmus* and *Epistemma*, but additional study is planned.

In Periplocoideae, pollinia are made up of loosely agglutinated tetrads (Verhoeven & Venter, 1998a). Tetrads may be fused (tectum and granular layers) in places where the pores of adjacent tetrads are touching.

Fusion between tetrad pores has not been observed in *Hemidesmus*. Verhoeven and Venter (1998a) observed wavy proximal tetrad walls in the pollinia of *Hemidesmus* and *Schlechterella* and postulated that this feature may assist in holding the tetrads together. These wavy tetrad walls were not observed in members of the Asian pollinial clade, *Epistemma*, or other Periplocoideae.

GENERAL CONCLUSIONS

The use of molecular evidence to elucidate evolutionary relationships can be particularly crucial in groups that exhibit a complex and widely varying array of floral forms such as Periplocoideae. The results of our analyses do not always agree with past generic or tribal level classifications; this may stem, at least in part, from an emphasis on rather homoplasious floral features by previous systematists. In agreement with previous studies, our results show that pollinia in Periplocoideae are likely non-homologous with those of Asclepiadoideae and Secamonoideae and support at least three origins of pollinia in Periplocoideae. We hope to clarify relationships and elucidate patterns of character evolution in this fascinating group through further sampling of taxa from throughout the subfamily, by completing sequencing of the plastid regions used in this study, and with more in-depth morphological investigations.

Literature Cited

- Arekal, G. D. & T. M. Ramakrishna. 1980. Pollen-carriers of Periplocaceae and their systematic value. *Proc. Indian Acad. Sci. Pl. Sci.* 89: 429–435.
- Bentham, G. 1876. Apocynaceae and Asclepiadaceae. Pp. 681–785 in G. Bentham & J. D. Hooker (editors), *Genera Plantarum*, 2(2). L. Reeve, London.
- Blattner, F. R. 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *BioTechniques* 27: 1180–1186.
- Browicz, K. 1966. The genus *Periploca* L. A monograph. *Arbor. Kórnickie* 11: 5–104.
- Brown, N. E. 1902. Asclepiadaceae. Pp. 233–234 in W. T. Thiselton-Dyer (editor), *Flora of Tropical Africa*, Vol. 4. L. Reeve, London.
- . 1904. Asclepiadaceae. Pp. 518–1036 in W. T. Thiselton-Dyer (editor), *Flora Capensis*, Vol. 4. L. Reeve, London.
- Brown, R. 1810. On the Asclepiadeae. London. Preprint of Brown, R. 1811. On the Asclepiadeae, a natural order of plants separated from the Apocineae of Jussieu. *Mem. Wern. Nat. Hist. Soc.* 1: 12–78.
- Bullock, A. A. 1956. Notes on African Asclepiadaceae VIII. *Kew Bull.* 1956: 503–522.
- . 1961. *Parquetina nigrescens*. *Kew Bull.* 15: 205–206.
- Civeyrel, L., A. le Thomas, K. Ferguson & M. W. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *matK* sequences. *Molec. Phylogen. Evol.* 9: 517–527.

- Demesure, B., N. Sodji & R. J. Petit. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molec. Ecol.* 4: 129–131.
- Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.
- Endress, M. E. & P. V. Bruyns. 2000. A revised classification of the Apocynaceae s.l. *Bot. Rev. (Lancaster)* 66: 1–56.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using bootstrap. *Evolution* 39: 783–791.
- Fishbein, M. 2001. Evolutionary innovation and diversification in the flowers of Asclepiadaceae. *Ann. Missouri Bot. Gard.* 88: 603–623.
- Forster, P. I. 1991. A taxonomic revision of *Gymnanthera* R. Br. (Asclepiadaceae: Periplocoideae) in Australia. *Austral. Syst. Bot.* 4: 563–569.
- . 1993. Conspectus of *Cryptolepis* R. Br. (Asclepiadaceae: Periplocoideae) in Maleisia. *Austrobaileya* 4: 67–73.
- Huelsenbeck, J. P., J. J. Bull & C. W. Cunningham. 1996. Combining data in phylogenetic analysis. *Trends Ecol. Evol.* 11: 152–158.
- Judd, W. S., R. W. Sanders & M. J. Donoghue. 1994. Angiosperm family pairs: Preliminary phylogenetic analyses. *Harvard Pap. Bot.* 1(5): 1–51.
- Klackenberg, J. 1997. Revision of the genus *Baroniella* Constantin & Gallaud (Asclepiadaceae, Periplocoidae). *Candollea* 52: 383–407.
- . 1998. Taxonomy and phylogeny of the genus *Camptocarpus* s.l. (Periplocoideae, Asclepiadaceae). *Bot. Jahrb. Syst.* 120: 45–85.
- . 1999. Revision of the Malagasy genera *Pentopetia* and *Ischnolepis* (Apocynaceae s.l., Periplocoideae). *Candollea* 54: 257–339.
- Kunze, H. 1993. Evolution of the translator in Periplocaceae and Asclepiadaceae. *Pl. Syst. Evol.* 185: 99–122.
- Lahaye, R., L. Civeyrel, T. Speck & N. Rowe. 2005. Evolution of shrub-like growth forms in the lianoid subfamily Secamonoideae (Apocynaceae s.l.) of Madagascar: Phylogeny, biomechanics, and development. *Amer. J. Bot.* 92: 1381–1396.
- Liede, S. 2001. Subtribe Astephaninae (Apocynaceae–Asclepiadoideae) reconsidered: New evidence based on cpDNA spacers. *Ann. Missouri Bot. Gard.* 88: 657–668.
- & F. Albers. 1994. Tribal disposition of genera in the Asclepiadaceae. *Taxon* 43: 201–231.
- Maddison, W. P. & D. R. Maddison. 1999. *MacClade: Analysis of Phylogeny and Character Evolution*. Version 3.08a. Sinauer, Sunderland, Massachusetts.
- Mason-Gamer, R. J. & E. A. Kellogg. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst. Biol.* 45: 524–545.
- Meve, U. & S. Liede. 2001. Reconsideration of the status of *Lavrania*, *Larryleachia* and *Notechidnopsis* (Asclepiadoideae–Ceropegieae). *S. African J. Bot.* 67: 161–168.
- & ———. 2002. A molecular phylogeny and generic rearrangement of the stapelioid Ceropegieae (Apocynaceae–Asclepiadoideae). *Pl. Syst. Evol.* 234: 171–209.
- & ———. 2001. Generic delimitations in tuberous Periplocoideae (Apocynaceae) from Africa and Madagascar. *Ann. Bot.* 93: 407–414.
- Nilsson, S., M. E. Endress & E. Grafström. 1993. On the relationship of the Apocynaceae and Periplocaceae. *Grana, Suppl.* 2: 3–20.
- Nishino, E. 1982. Corolla tube formation in six species of Apocynaceae. *Bot. Mag. (Tokyo)* 95: 1–17.
- Omlor, R. 1996. Do *Menabea venenata* and *Secamonopsis madagascariensis* represent missing links between Periplocaceae, Secomonoideae and Mersdeniaceae (Asclepiadaceae)? *Kew Bull.* 51: 695–715.
- Potgieter, K. & V. A. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trnL* intron and *trnL-F* spacer sequences and propagule characters. *Ann. Missouri Bot. Gard.* 88: 523–549.
- Rambaut, A. 1996. Se-Al: Sequence Alignment Editor. Available at: <http://evolve.zoo.ox.ac.uk/>.
- Safwat, F. M. 1962. The floral morphology of *Secamone* and the evolution of the pollinating apparatus in Asclepiadaceae. *Ann. Missouri Bot. Gard.* 49: 95–129.
- Schick, B. 1982. Zur Morphologie, Entwicklung, Feinstruktur und Funktion des Translators von *Periploca* L. (Asclepiadaceae). *Trop. Subtrop. Pflanzenwelt* 40: 513–553.
- Schlechter, F. R. R. 1905. Periplocaceae. Pp. 351–353 in K. Schumann & K. Lauterbach (editors), *Nachträge zur Flora des Deutschen Schutzgebiete in der Südsee*. Borntraeger, Leipzig.
- Schumann, K. 1895. Apocynaceae and Asclepiadaceae. Pp. 109–305 in A. Engler & K. Prantl (editors), *Die Natürlichen Pflanzenfamilien*, Vol. 4(2). Engelmann, Leipzig.
- Sennblad, B. & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbcL* data. *Pl. Syst. Evol.* 202: 153–175.
- Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling & R. L. Small. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer. J. Bot.* 92: 142–166.
- Soltis, D. E., R. K. Kuzoff, M. E. Mort, M. Zanis, M. Fishbein, L. Hufford, J. Koontz & M. K. Arroyo. 2001. Elucidating deep-level phylogenetic relationships in Saxifragaceae using sequences for six chloroplastic and nuclear DNA regions. *Ann. Missouri Bot. Gard.* 88: 669–693.
- Sun, Y., D. Z. Skinner, G. H. Liang & S. H. Hulbert. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theor. Appl. Genet.* 89: 26–32.
- Swarupanandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. Chand Basha. 1996. The subfamilial and tribal classification of the family Asclepiadaceae. *Bot. J. Linn. Soc.* 120: 327–369.
- Swofford, D. L. 1999. *PAUP*: Phylogenetic analysis using parsimony (*and Other Methods)*, Vers. 4.0b10. Sinauer, Sunderland, Massachusetts.
- Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.
- Venter, H. J. T. 1997. A revision of *Periploca* (Periplocaceae). *S. African J. Bot.* 63: 123–128.
- & R. L. Verhoeven. 1988. *Raphionacme lobulata* (Periplocoidae), a new species from the Eastern Cape Province, South Africa. *S. African J. Bot.* 54: 603–606.
- & ———. 1996. The 'minor confusion' around *Parquetina nigrescens* (Periplocaceae). *S. African J. Bot.* 62: 23–30.
- & ———. 1997. A tribal classification of the Periplocoideae (Apocynaceae). *Taxon* 46: 705–720.

-
- & ———. 2001. Diversity and relationships within the Periplocoideae (Apocynaceae). *Ann. Missouri Bot. Gard.* 88: 550–568.
- , A. P. Dold, R. L. Verhoeven & G. Ionta. 2006. *Kappia lobulata* (Apocynaceae, Periplocoideae), a new genus from South Africa. *S. African J. Bot.* 72: 529–533.
- Verhoeven, R. L. & H. J. T. Venter. 1988. Pollen morphology of *Raphionacme* (Periplocaceae). *S. African J. Bot.* 54: 123–132.
- & ———. 1989. Pollen morphology of *Petopentia* and *Tacazzea* (Periplocoideae). *S. African J. Bot.* 55: 207–214.
- & ———. 1994a. Pollen morphology of *Periploca* (Periplocaceae). *S. African J. Bot.* 60: 198–202.
- & ———. 1994b. Pollen morphology of the Periplocaceae from Madagascar. *Grana* 33: 295–308.
- & ———. 1997. The translator in *Raphionacme* (Periplocaceae). *S. African J. Bot.* 63: 46–54.
- & ———. 1998a. Pollinium structure in Periplocoideae (Apocynaceae). *Grana* 37: 1–14.
- & ———. 1998b. Pollinium in *Schlechterella*: Periplocoideae (Apocynaceae). *S. African J. Bot.* 64: 256–257.
- & ———. 2001. Pollen morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae). *Ann. Missouri Bot. Gard.* 88: 569–582.
- & ———. 2002. Pollinium structure in *Epistemma*: Periplocoideae (Apocynaceae). *S. African J. Bot.* 68: 401–403.
- , ——— & J. D. S. Kotze. 1989. Pollen morphology of *Petopentia* and *Tacazzea* (Periplocaceae). *S. African J. Bot.* 55: 207–214.